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**Baichwal et al.**

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(54) **RIP: NOVEL HUMAN PROTEIN INVOLVED  
IN TUMOR NECROSIS FACTOR SIGNAL  
TRANSDUCTION**

(52) **U.S. Cl.** ..... **530/350**; 435/194; 436/501  
(58) **Field of Search** ..... 530/351, 350,  
530/395; 425/85.7; 514/2, 8; 435/194; 436/501

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(56) **References Cited**

**U.S. PATENT DOCUMENTS**

5,674,734 \* 10/1997 Leder et al. .... 435/252.3

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(US)

\* cited by examiner

(\* ) **Notice:** Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.

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(21) **Appl. No.:** **09/132,118**

(57) **ABSTRACT**

(22) **Filed:** **Aug. 11, 1998**

The invention relates to a human Receptor Interacting  
Protein (hRIP), nucleic acids which encode hRIP and meth-  
ods of using the subject compositions; in particular, methods  
such as hRIP-based in vitro binding assays and phosphory-  
lation assays for screening chemical libraries for lead com-  
pounds for pharmacological agents.

**Related U.S. Application Data**

(63) Continuation-in-part of application No. 08/553,727, filed on  
Oct. 23, 1995, now abandoned.

(51) **Int. Cl.**<sup>7</sup> ..... **C07K 14/435**

**24 Claims, No Drawings**

**RIP: NOVEL HUMAN PROTEIN INVOLVED  
IN TUMOR NECROSIS FACTOR SIGNAL  
TRANSDUCTION**

**CROSS-REFERENCE TO RELATED  
APPLICATION**

This is a CIP of claims priority under 35USC120 to U.S. patent application Ser. No. 08/553,727, filed Oct. 23, 1995 now abandoned.

**INTRODUCTION**

1. Field of the Invention

The field of this invention is a novel human kinase involved in tumor necrosis factor signal transduction and its use in drug screening.

2. Background

Tumor necrosis factor (TNF) is an important cytokine involved in the signaling of a number of cellular responses including cytotoxicity, anti-viral activity, immun.-regulatory activities and the transcriptional regulation of a number of genes. The TNF receptors (TNF-R1 and TNF-R2) are members of the larger TNF receptor superfamily which also includes the Fas antigen, CD27, CD30, CD40, and the low affinity nerve growth factor receptor. Members of this family have been shown to participate in a variety of biological properties, including programmed cell death, antiviral activity and activation of the transcription factor NF- $\kappa$ B in a wide variety of cell types.

Accordingly, it is desired to identify agents which specifically modulate transduction of TNF receptor family signaling. Unfortunately, the components of the signaling pathway remain largely unknown; hence, the reagents necessary for the development of high-throughput screening assays for such therapeutics are unavailable. Elucidation of TNF receptor family signal transduction pathways leading to NF- $\kappa$ B activation would provide valuable insight into mechanisms to alleviate inflammation. In particular, components of this pathway would provide valuable targets for automated, cost-effective, high throughput drug screening and hence would have immediate application in a broad range of domestic and international pharmaceutical and biotechnology drug development programs.

Relevant Literature

Stanger et al. (1995) Cell 81, 513–523 report the existence of a Receptor Interacting Protein (RIP) and its functional expression. VanArsdale and Ware (1994) J Immunology 153:3043–3050 describe proteins associated with TNF-R1. The cloning and amino acid sequencing of TNF-R1 is disclosed in Schall et al (1990) Cell 61, 361 and Loetscher et al (1990) Cell 61, 351; the identification of a “death domain” in TNF-R1 is disclosed in Tartaglia et al. (1993) Cell 74:845–853. The cloning and amino acid sequence of a TNF-R associated death domain protein (TRADD) is described by Hsu et al. (1995) Cell 81, 495–504. The cloning and amino acid sequence of the Fas antigen is disclosed in Itoh et al (1991) Cell 66, 233–243. For a recent review, see Smith et al. (1994) Cell 76:959–962 and Vandenabelle et al. (1995) Trends Cell Biol. 5, 392–399.

**SUMMARY OF THE INVENTION**

The invention provides methods and compositions relating to a human Receptor Interacting Protein (hRIP). The compositions include nucleic acids which encode hRIP, hRIP kinase domains, and recombinant proteins made from these nucleic acids. The invention also provides methods for

screening chemical libraries for lead compounds for a pharmacological agent useful in the diagnosis or treatment of disease associated hRIP activity or hRIP-dependent signal transduction. In one embodiment, the methods involve incubating a mixture of hRIP, a natural intracellular hRIP substrate or binding target and a candidate pharmacological agent and determining if the presence of the agent modulates the ability of hRIP to selectively phosphorylate the substrate or bind the binding target. Specific agents provide lead compounds for pharmacological agents capable of disrupting hRIP function.

**DETAILED DESCRIPTION OF THE  
INVENTION**

A human RIP-encoding nucleic acid sequence is set out in SEQ ID NO: 1. A human RIP kinase domain-encoding nucleic acid sequence is set out in SEQ ID NO: 1, nucleotides 1–900. A human RIP amino acid sequence is set out in SEQ ID NO: 2; and a hRIP kinase domain sequence is set out in SEQ ID NO:2, residues 1–300.

Natural nucleic acids encoding hRIP are readily isolated from cDNA libraries with PCR primers and hybridization probes containing portions of the nucleic acid sequence of SEQ ID NO:1. For example, we used low stringency hybridization at 42° C. (hybridization buffer: 20% formamide, 10% Denhardt, 0.5% SDS, 5 $\times$ SSPE; with membrane washes at room temperature with 5 $\times$ SSPE/0.5% SDS) with a 120 base oligonucleotide probe (SEQ ID NO: 1, nucleotides 1728–1847) to isolate a native human RIP cDNA from a library prepared from human umbilical vein endothelial cells. In addition, synthetic hRIP-encoding nucleic acids may be generated by automated synthesis.

The subject nucleic acids are recombinant, meaning they comprise a sequence joined to a nucleotide other than that to which sequence is naturally joined and isolated from a natural environment. The nucleic acids may be part of hRIP-expression vectors and may be incorporated into cells for expression and screening, transgenic animals for functional studies (e.g. the efficacy of candidate drugs for disease associated with expression of a hRIP), etc. These nucleic acids find a wide variety of applications including use as templates for transcription, hybridization probes, PCR primers, therapeutic nucleic acids, etc.; use in detecting the presence of hRIP genes and gene transcripts, in detecting or amplifying nucleic acids encoding additional hRIP homologs and structural analogs, and in gene therapy applications.

In a particular embodiment, the invention provides RIP-Thr<sup>514</sup> polypeptides, RIP-Thr<sup>514</sup> polypeptide-encoding nucleic acids/polynucleotides, and RIP-Thr<sup>514</sup> polypeptide-based methods (below), which RIP-Thr<sup>514</sup> polypeptides comprise at least 8, preferably at least 10, more preferably at least 12, more preferably at least 16, most preferably at least 24 consecutive amino acid residues of the amino acid sequence set forth as SEQ ID NO:2, which consecutive amino acid residues comprise the amino acid residue 514 (Thr) of SEQ ID NO:2. Exemplary RIP-Thr<sup>514</sup> polypeptides having RIP-Thr<sup>514</sup> binding specificity and immunologically distinguishable from RIP-Ser<sup>514</sup> are shown in Table I.

TABLE I. Exemplary RIP-Thr<sup>514</sup> Polypeptides Having RIP-Thr<sup>514</sup> Binding Specificity

$\alpha$ 1 (SEQ ID NO:2, residues 509–518)  
 $\alpha$ 2 (SEQ ID NO:2, residues 514–521)  
 $\alpha$ 3 (SEQ ID NO:2, residues 506–514)  
 $\alpha$ 4 (SEQ ID NO:2, residues 504–524)  
 $\alpha$ 5 (SEQ ID NO:2, residues 498–514)

$\alpha\Delta 6$  (SEQ ID NO:2, residues 514–534)  
 $\alpha\Delta 7$  (SEQ ID NO:2, residues 513–520)  
 $\alpha\Delta 8$  (SEQ ID NO:2, residues 508–515)  
 $\alpha\Delta 9$  (SEQ ID NO:2, residues 512–522)  
 $\alpha\Delta 10$  (SEQ ID NO:2, residues 423–514)  
 $\alpha\Delta 11$  (SEQ ID NO:2, residues 423–543)  
 $\alpha\Delta 12$  (SEQ ID NO:2, residues 423–579)  
 $\alpha\Delta 13$  (SEQ ID NO:2, residues 423–633)  
 $\alpha\Delta 14$  (SEQ ID NO:2, residues 423–671)  
 $\alpha\Delta 15$  (SEQ ID NO:2, residues 514–543)  
 $\alpha\Delta 16$  (SEQ ID NO:2, residues 514–579)  
 $\alpha\Delta 17$  (SEQ ID NO:2, residues 514–633)  
 $\alpha\Delta 18$  (SEQ ID NO:2, residues 514–671)

In a particular embodiment, the invention provides RIP-ACA<sup>1540-1542</sup> polynucleotides, comprising at least 18, 24, 36, 48, 72, 148, 356 or 728 consecutive nucleotides of the nucleotide sequence set forth as SEQ ID NO:1, which consecutive polynucleotides comprise the polynucleotides 1540–1542 (ACA) of SEQ ID NO:1. Exemplary RIP-ACA<sup>1540-1542</sup> polynucleotides and allele specific oligonucleotide probes having RIP-ACA<sup>1540-1542</sup> binding specificity and distinguishable by hybridization assays from RIP-TCT<sup>1540-1542</sup> are shown in Table II.

TABLE II. Exemplary RIP-ACA<sup>1540-1542</sup> Polynucleotides Having RIP-ACA<sup>1540-1542</sup> Binding Specificity

$\alpha\Delta 1$  (SEQ ID NO:1, nucleotides 1540–1557)  
 $\alpha\Delta 2$  (SEQ ID NO:1, nucleotides 1540–1563)  
 $\alpha\Delta 3$  (SEQ ID NO:1, nucleotides 1540–1675)  
 $\alpha\Delta 4$  (SEQ ID NO:1, nucleotides 1540–1699)  
 $\alpha\Delta 5$  (SEQ ID NO:1, nucleotides 1525–1542)  
 $\alpha\Delta 6$  (SEQ ID NO:1, nucleotides 1519–1542)  
 $\alpha\Delta 7$  (SEQ ID NO:1, nucleotides 1507–1542)  
 $\alpha\Delta 8$  (SEQ ID NO:1, nucleotides 1483–1542)  
 $\alpha\Delta 9$  (SEQ ID NO:1, nucleotides 1537–1545)  
 $\alpha\Delta 10$  (SEQ ID NO:1, nucleotides 1534–1548)  
 $\alpha\Delta 11$  (SEQ ID NO:1, nucleotides 1528–1554)  
 $\alpha\Delta 12$  (SEQ ID NO:1, nucleotides 1516–1566)  
 $\alpha\Delta 13$  (SEQ ID NO:1, nucleotides 1504–1554)  
 $\alpha\Delta 14$  (SEQ ID NO:1, nucleotides 1492–1568)

The invention provides efficient methods of identifying pharmacological agents or lead compounds for agents active at the level of a hRIP modulatable cellular function, particularly hRIP mediated TNF receptor or Tumor necrosis factor receptor associated Factor-2 (TRAF2) or TRADD-induced signal transduction. For example, we have found that a binding complex comprising TNF R1, TRADD, and hRIP exists in TNF-stimulated cells. Generally, the screening methods involve assaying for compounds which interfere with a hRIP activity such as kinase activity or TRAF2 or TRADD binding. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development. Target therapeutic indications are limited only in that the target cellular function be subject to modulation, usually inhibition, by disruption of the formation of a complex comprising hRIP and one or more natural hRIP intracellular binding targets including substrates or otherwise modulating hRIP kinase activity. Target indications may include infection, genetic disease, cell growth and regulatory or immunologic dysfunction, such as neoplasia, inflammation, hypersensitivity, etc.

A wide variety of assays for binding agents are provided including labeled *in vitro* kinase assays, protein-protein

binding assays, immunoassays, cell based assays, etc. The hRIP compositions used in the methods are recombinantly produced from nucleic acids having the disclosed hRIP nucleotide sequences. The hRIP may be part of a fusion product with another peptide or polypeptide, e.g. a polypeptide that is capable of providing or enhancing protein-protein binding, stability under assay conditions (e.g. a tag for detection or anchoring), etc.

The assay mixtures comprise one or more natural intracellular hRIP binding targets including substrates, such as TRADD, TRAF2, or, in the case of an autophosphorylation assay, the hRIP itself can function as the binding target. In one embodiment, the mixture comprises a complex of hRIP, TRADD and TNFR1. A hRIP derived pseudosubstrate may be used or modified (e.g. A to S/T substitutions) to generate effective substrates for use in the subject kinase assays as can synthetic peptides or other protein substrates. Generally, hRIP-specificity of the binding agent is shown by kinase activity (i.e. the agent demonstrates activity of an hRIP substrate, agonist, antagonist, etc.) or binding equilibrium constants (usually at least about  $10^6 \text{ M}^{-1}$ , preferably at least about  $10^8 \text{ M}^{-1}$ , more preferably at least about  $10^9 \text{ M}^{-1}$ ). A wide variety of cell-based and cell-free assays may be used to demonstrate hRIP-specific binding; preferred are rapid *in vitro*, cell-free assays such as mediating or inhibiting hRIP-protein (e.g. hRIP-TRADD) binding, phosphorylation assays, immunoassays, etc.

The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds; preferably small organic compounds and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like salts, buffers, neutral proteins, e.g. albumin, detergents, etc. which may be used to facilitate optimal binding and/or reduce non-specific or background interactions, etc. Also, reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, antimicrobial agents, etc. may be used.

In a preferred *in vitro*, binding assay, a mixture of at least the kinase domain of hRIP, one or more binding targets or substrates and the candidate agent is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the hRIP specifically binds the cellular binding target at a first binding affinity or phosphorylates the substrate at a first rate. After incubation, a second binding affinity or rate is detected. Detection may be effected in any convenient way. For cell-free binding assays, one of the components usually comprises or is coupled to a label. The label may provide for direct detection as radioactivity, luminescence, optical or electron density, etc. or indirect detection such as an epitope tag, an enzyme, etc. A variety of methods may be used to detect the label depending on the nature of the label and other assay components. For example, the label may be detected bound to the solid substrate or a portion of the bound complex containing the label may be separated from the solid substrate, and thereafter the label detected.

The following examples are offered by way of illustration and not by way of limitation.

#### EXAMPLES

##### 1. Protocol for hRIP Autophosphorylation Assay

###### A. Reagents:

- Neutralite Avidin: 20  $\mu\text{g/ml}$  in PBS.
- hRIP:  $10^{-8}$ – $10^{-5}$  M biotinylated hRIP kinase domain, residues 1–300 at 20  $\mu\text{g/ml}$  in PBS.

## 5

- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.
- $[^{32}\text{P}]\gamma\text{-ATP}$  10xstock:  $2 \times 10^{-5}$  M cold ATP with 100  $\mu\text{Ci}$   $[^{32}\text{P}]\gamma\text{-ATP}$ . Place in the 4° C. microfridge during screening.
- Protease inhibitor cocktail (1000x): 10 mg Trypsin Inhibitor (BMB #109894), 10 mg Aprotinin (BMB #236624), 25 mg Benzamidine (Sigma #B-6506), 25 mg Leupeptin (BMB #1017128), 10 mg APMSF (BMB #917575), and 2 mM  $\text{NaVO}_3$  (Sigma #S-6508) in 10 ml PBS.
- B. Preparation of assay plates:
- Coat with 120  $\mu\text{l}$  of stock Neutralite avidin per well overnight at 4° C.
- Wash 2 times with 200  $\mu\text{l}$  PBS.
- Block with 150  $\mu\text{l}$  of blocking buffer.
- Wash 2 times with 200  $\mu\text{l}$  PBS.
- C. Assay:
- Add 40  $\mu\text{l}$  assay buffer/well.
- Add 40  $\mu\text{l}$  biotinylated hRIP (0.1–10 pmoles/40  $\mu\text{l}$  in assay buffer)
- Add 10  $\mu\text{l}$  compound or extract.
- Add 10  $\mu\text{l}$   $[^{32}\text{P}]\gamma\text{-ATP}$  10xstock.
- Shake at 30° C. for 15 minutes.
- Incubate additional 45 minutes at 30° C.
- Stop the reaction by washing 4 times with 200  $\mu\text{l}$  PBS.
- Add 150  $\mu\text{l}$  scintillation cocktail.
- Count in Topcount.
- D. Controls for all assays (located on each plate):
- Non-specific binding (no RIP added)
  - cold ATP to achieve 80% inhibition.
2. Protocol for hRIP—Substrate Phosphorylation Assay
- A. Reagents:
- Neutralite Avidin: 20  $\mu\text{g}/\text{ml}$  in PBS.
- hRIP:  $10^{-8}$ – $10^{-5}$  M hRIP at 20  $\mu\text{g}/\text{ml}$  in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.
- $[^{32}\text{P}]\gamma\text{-ATP}$  10xstock:  $2 \times 10^{-5}$  M cold ATP with 100  $\mu\text{Ci}$   $[^{32}\text{P}]\gamma\text{-ATP}$ . Place in the 4° C. microfridge during screening.
- Substrate:  $2 \times 10^{-6}$  M biotinylated synthetic peptide kinase substrate at 20  $\mu\text{g}/\text{ml}$  in PBS.
- Protease inhibitor cocktail (1000x): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB #236624), 25 mg Benzamidine (Sigma #B-6506), 25 mg Leupeptin (BMB #1017128), 10 mg APMSF (BMB #917575), and 2 mM  $\text{NaVO}_3$  (Sigma #S-6508) in 10 ml PBS.
- B. Preparation of assay plates:
- Coat with 120  $\mu\text{l}$  of stock Neutralite avidin per well overnight at 4° C.
- Wash 2 times with 200  $\mu\text{l}$  PBS.
- Block with 150  $\mu\text{l}$  of blocking buffer.
- Wash 2 times with 200  $\mu\text{l}$  PBS.
- C. Assay:
- Add 40  $\mu\text{l}$  assay buffer/well.
- Add 10  $\mu\text{l}$  compound or extract.
- Add 10  $\mu\text{l}$   $^{32}\text{P}$ -RIP (20,000–25,000 cpm/0.1–10 pmoles/well= $10^{-9}$ – $10^{-7}$  M final concentration).
- Shake at 25° C. for 15 minutes.
- Incubate additional 45 minutes at 25° C.
- Add 40  $\mu\text{l}$  eptide-tagged TRADD (0.1–10 pmoles/40  $\mu\text{l}$  in assay buffer)
- Incubate 1 hour at room temperature.
- Stop the reaction by washing 4 times with 200  $\mu\text{l}$  PBS.
- Add 150  $\mu\text{l}$  scintillation cocktail.
- Count in Topcount.
- D. Controls for all assays (located on each plate):
- Non-specific binding (no hRIP added)
  - Soluble (non-tagged TRADD) to achieve 80% inhibition.
4. Protocol for hRIP—TRAF2 Binding Assay
- A. Reagents:
- Anti-myc antibody: 20  $\mu\text{g}/\text{ml}$  in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.

## 6

- Add 40  $\mu\text{l}$  hRIP (0.1–10 pmoles/40  $\mu\text{l}$  in assay buffer)
- Add 10  $\mu\text{l}$  compound or extract.
- Shake at 30° C. for 15 minutes.
- Add 10  $\mu\text{l}$   $[^{32}\text{P}]\gamma\text{-ATP}$  10xstock.
- Add 10  $\mu\text{l}$  substrate.
- Shake at 30° C. for 15 minutes.
- Incubate additional 45 minutes at 30° C.
- Stop the reaction by washing 4 times with 200  $\mu\text{l}$  PBS.
- Add 150  $\mu\text{l}$  scintillation cocktail.
- Count in Topcount.
- D. Controls for all assays (located on each plate):
- Non-specific binding (no RIP added)
  - cold ATP to achieve 80% inhibition.
3. Protocol for hRIP—TRADD Binding Assay
- A. Reagents:
- Anti-myc antibody: 20  $\mu\text{g}/\text{ml}$  in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.
- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM  $\beta$ -mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.
- $^{33}\text{P}$  hRIP 10xstock:  $10^{-8}$ – $10^{-6}$  M “cold” hRIP (full length) supplemented with 200,000–250,000 cpm of labeled hRIP (HMK-tagged) (Beckman counter). Place in the 4° C. microfridge during screening.
- Protease inhibitor cocktail (1000x): 10 mg Trypsin Inhibitor (BMB #109894), 10 mg Aprotinin (BMB #236624), 25 mg Benzamidine (Sigma #B-6506), 25 mg Leupeptin (BMB #1017128), 10 mg APMSF (BMB #917575), and 2 mM  $\text{NaVO}_3$  (Sigma #S-6508) in 10 ml PBS.
- TRADD:  $10^{-8}$ – $10^{-5}$  M myc eptide-tagged TRADD in PBS.
- B. Preparation of assay plates:
- Coat with 120  $\mu\text{l}$  of stock anti-myc antibody per well overnight at 4° C.
- Wash 2x with 200  $\mu\text{l}$  PBS.
- Block with 150  $\mu\text{l}$  of blocking buffer.
- Wash 2x with 200  $\mu\text{l}$  PBS.
- C. Assay:
- Add 40  $\mu\text{l}$  assay buffer/well.
- Add 10  $\mu\text{l}$  compound or extract.
- Add 10  $\mu\text{l}$   $^{33}\text{P}$ -RIP (20,000–25,000 cpm/0.1–10 pmoles/well= $10^{-9}$ – $10^{-7}$  M final concentration).
- Shake at 25° C. for 15 minutes.
- Incubate additional 45 minutes at 25° C.
- Add 40  $\mu\text{l}$  eptide-tagged TRADD (0.1–10 pmoles/40  $\mu\text{l}$  in assay buffer)
- Incubate 1 hour at room temperature.
- Stop the reaction by washing 4 times with 200  $\mu\text{l}$  PBS.
- Add 150  $\mu\text{l}$  scintillation cocktail.
- Count in Topcount.
- D. Controls for all assays (located on each plate):
- Non-specific binding (no hRIP added)
  - Soluble (non-tagged TRADD) to achieve 80% inhibition.
4. Protocol for hRIP—TRAF2 Binding Assay
- A. Reagents:
- Anti-myc antibody: 20  $\mu\text{g}/\text{ml}$  in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.

Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM  $\beta$ -mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.

$^{33}\text{P}$  hRIP 10 $\times$ stock:  $10^{-8}$ – $10^{-6}$  M “cold” hRIP kinase domain, residues 1–300, supplemented with 200,000–250,000 cpm of labeled hRIP kinase domain (HMK-tagged) (Beckman counter). Place in the 4° C. microfridge during screening.

Protease inhibitor cocktail (1000 $\times$ ): 10 mg Trypsin Inhibitor (BMB #109894), 10 mg Aprotinin (BMB #236624), 25 mg Benzamidine (Sigma #B-6506), 25 mg Leupeptin (BMB #1017128), 10 mg APMSF (BMB #917575), and 2 mM  $\text{NaVO}_3$  (Sigma #S-6508) in 10 ml PBS.

TRAF2:  $10^{-8}$ – $10^{-5}$  M myc epitope-tagged TRAF2 in PBS.

#### B. Preparation of assay plates:

Coat with 120  $\mu\text{l}$  of stock anti-myc antibody per well overnight at 4° C.

Wash 2 $\times$  with 200  $\mu\text{l}$  PBS.

Block with 150  $\mu\text{l}$  of blocking buffer.

Wash 2 $\times$  with 200  $\mu\text{l}$  PBS.

#### C. Assay:

Add 40  $\mu\text{l}$  assay buffer/well.

Add 10  $\mu\text{l}$  compound or extract.

Add 10  $\mu\text{l}$   $^{33}\text{P}$ -RIP kinase domain (20,000–25,000 cpm/0.1–10 pmoles/well= $10^{-9}$ – $10^{-7}$  M final concentration).

Shake at 25° C. for 15 minutes.

Incubate additional 45 minutes at 25° C.

Add 40  $\mu\text{l}$  epitope-tagged TRAF2 (0.1–10 pmoles/40  $\mu\text{l}$  in assay buffer)

Incubate 1 hour at room temperature.

Stop the reaction by washing 4 times with 200  $\mu\text{l}$  PBS.

Add 150  $\mu\text{l}$  scintillation cocktail.

Count in Topcount.

#### D. Controls for all assays (located on each plate):

a. Non-specific binding (no hRIP kinase domain added)

b. Soluble (non-tagged TRAF2) to achieve 80% inhibition.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

### SEQUENCE LISTING

#### (1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 2

#### (2) INFORMATION FOR SEQ ID NO:1:

##### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2016 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

##### (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1..2013

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG CAA CCA GAC ATG TCC TTG AAT GTC ATT AAG ATG AAA TCC AGT GAC	48
Met Gln Pro Asp Met Ser Leu Asn Val Ile Lys Met Lys Ser Ser Asp	
1 5 10 15	
TTC CTG GAG AGT GCA GAA CTG GAC AGC GGA GGC TTT GGG AAG GTG TCT	96
Phe Leu Glu Ser Ala Glu Leu Asp Ser Gly Gly Phe Gly Lys Val Ser	
20 25 30	
CTG TGT TTC CAC AGA ACC CAG GGA CTC ATG ATC ATG AAA ACA GTG TAC	144
Leu Cys Phe His Arg Thr Gln Gly Leu Met Ile Met Lys Thr Val Tyr	
35 40 45	
AAG GGG CCC AAC TGC ATT GAG CAC AAC GAG GCC CTC TTG GAG GAG GCG	192
Lys Gly Pro Asn Cys Ile Glu His Asn Glu Ala Leu Leu Glu Glu Ala	
50 55 60	
AAG ATG ATG AAC AGA CTG AGA CAC AGC CGG GTG GTG AAG CTC CTG GGC	240
Lys Met Met Asn Arg Leu Arg His Ser Arg Val Val Lys Leu Leu Gly	
65 70 75 80	

-continued

GTC	ATC	ATA	GAG	GAA	GGG	AAG	TAC	TCC	CTG	GTG	ATG	GAG	TAC	ATG	GAG	288
Val	Ile	Ile	Glu	Glu	Gly	Lys	Tyr	Ser	Leu	Val	Met	Glu	Tyr	Met	Glu	
				85					90					95		
AAG	GGC	AAC	CTG	ATG	CAC	GTG	CTG	AAA	GCC	GAG	ATG	AGT	ACT	CCG	CTT	336
Lys	Gly	Asn	Leu	Met	His	Val	Leu	Lys	Ala	Glu	Met	Ser	Thr	Pro	Leu	
			100					105					110			
TCT	GTA	AAA	GGA	AGG	ATA	ATT	TTG	GAA	ATC	ATT	GAA	GGA	ATG	TGC	TAC	384
Ser	Val	Lys	Gly	Arg	Ile	Ile	Leu	Glu	Ile	Ile	Glu	Gly	Met	Cys	Tyr	
		115					120					125				
TTA	CAT	GGA	AAA	GGC	GTG	ATA	CAC	AAG	GAC	CTG	AAG	CCT	GAA	AAT	ATC	432
Leu	His	Gly	Lys	Gly	Val	Ile	His	Lys	Asp	Leu	Lys	Pro	Glu	Asn	Ile	
	130					135					140					
CTT	GTT	GAT	AAT	GAC	TTC	CAC	ATT	AAG	ATC	GCA	GAC	CTC	GGC	CTT	GCC	480
Leu	Val	Asp	Asn	Asp	Phe	His	Ile	Lys	Ile	Ala	Asp	Leu	Gly	Leu	Ala	
145					150					155					160	
TCC	TTT	AAG	ATG	TGG	AGC	AAA	CTG	AAT	AAT	GAA	GAG	CAC	AAT	GAG	CTG	528
Ser	Phe	Lys	Met	Trp	Ser	Lys	Leu	Asn	Asn	Glu	Glu	His	Asn	Glu	Leu	
			165					170					175			
AGG	GAA	GTG	GAC	GGC	ACC	GCT	AAG	AAG	AAT	GGC	GGC	ACC	CTC	TAC	TAC	576
Arg	Glu	Val	Asp	Gly	Thr	Ala	Lys	Lys	Asn	Gly	Gly	Thr	Leu	Tyr	Tyr	
			180					185					190			
ATG	GCG	CCC	GAG	CAC	CTG	AAT	GAC	GTC	AAC	GCA	AAG	CCC	ACA	GAG	AAG	624
Met	Ala	Pro	Glu	His	Leu	Asn	Asp	Val	Asn	Ala	Lys	Pro	Thr	Glu	Lys	
		195					200					205				
TCG	GAT	GTG	TAC	AGC	TTT	GCT	GTA	GTA	CTC	TGG	GCG	ATA	TTT	GCA	AAT	672
Ser	Asp	Val	Tyr	Ser	Phe	Ala	Val	Val	Leu	Trp	Ala	Ile	Phe	Ala	Asn	
	210					215					220					
AAG	GAG	CCA	TAT	GAA	AAT	GCT	ATC	TGT	GAG	CAG	CAG	TTG	ATA	ATG	TGC	720
Lys	Glu	Pro	Tyr	Glu	Asn	Ala	Ile	Cys	Glu	Gln	Gln	Leu	Ile	Met	Cys	
225					230					235					240	
ATA	AAA	TCT	GGG	AAC	AGG	CCA	GAT	GTG	GAT	GAC	ATC	ACT	GAG	TAC	TGC	768
Ile	Lys	Ser	Gly	Asn	Arg	Pro	Asp	Val	Asp	Asp	Ile	Thr	Glu	Tyr	Cys	
			245						250					255		
CCA	AGA	GAA	ATT	ATC	AGT	CTC	ATG	AAG	CTC	TGC	TGG	GAA	GCG	AAT	CCG	816
Pro	Arg	Glu	Ile	Ile	Ser	Leu	Met	Lys	Leu	Cys	Trp	Glu	Ala	Asn	Pro	
			260					265					270			
GAA	GCT	CGG	CCG	ACA	TTT	CCT	GGC	ATT	GAA	GAA	AAA	TTT	AGG	CCT	TTT	864
Glu	Ala	Arg	Pro	Thr	Phe	Pro	Gly	Ile	Glu	Glu	Lys	Phe	Arg	Pro	Phe	
		275					280					285				
TAT	TTA	AGT	CAA	TTA	GAA	GAA	AGT	GTA	GAA	GAG	GAC	GTG	AAG	AGT	TTA	912
Tyr	Leu	Ser	Gln	Leu	Glu	Glu	Ser	Val	Glu	Glu	Asp	Val	Lys	Ser	Leu	
	290					295					300					
AAG	AAA	GAG	TAT	TCA	AAC	GAA	AAT	GCA	GTT	GTG	AAG	AGA	ATG	CAG	TCT	960
Lys	Lys	Glu	Tyr	Ser	Asn	Glu	Asn	Ala	Val	Val	Lys	Arg	Met	Gln	Ser	
305					310					315					320	
CTT	CAA	CTT	GAT	TGT	GTG	GCA	GTA	CCT	TCA	AGC	CGG	TCA	AAT	TCA	GCC	1008
Leu	Gln	Leu	Asp	Cys	Val	Ala	Val	Pro	Ser	Ser	Arg	Ser	Asn	Ser	Ala	
			325						330					335		
ACA	GAA	CAG	CCT	GGT	TCA	CTG	CAC	AGT	TCC	CAG	GGA	CTT	GGG	ATG	GGT	1056
Thr	Glu	Gln	Pro	Gly	Ser	Leu	His	Ser	Ser	Gln	Gly	Leu	Gly	Met	Gly	
			340					345					350			
CCT	GTG	GAG	GAG	TCC	TGG	TTT	GCT	CCT	TCC	CTG	GAG	CAC	CCA	CAA	GAA	1104
Pro	Val	Glu	Glu	Ser	Trp	Phe	Ala	Pro	Ser	Leu	Glu	His	Pro	Gln	Glu	
		355					360					365				
GAG	AAT	GAG	CCC	AGC	CTG	CAG	AGT	AAA	CTC	CAA	GAC	GAA	GCC	AAC	TAC	1152
Glu	Asn	Glu	Pro	Ser	Leu	Gln	Ser	Lys	Leu	Gln	Asp	Glu	Ala	Asn	Tyr	
		370				375					380					
CAT	CTT	TAT	GGC	AGC	CGC	ATG	GAC	AGG	CAG	ACG	AAA	CAG	CAG	CCC	AGA	1200
His	Leu	Tyr	Gly	Ser	Arg	Met	Asp	Arg	Gln	Thr	Lys	Gln	Gln	Pro	Arg	
				385		390				395					400	

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CAG AAT GTG GCT TAC AAC AGA GAG GAG GAA AGG AGA CGC AGG GTC TCC	1248
Gln Asn Val Ala Tyr Asn Arg Glu Glu Glu Arg Arg Arg Arg Val Ser	
405 410 415	
CAT GAC CCT TTT GCA CAG CAA AGA CCT TAC GAG AAT TTT CAG AAT ACA	1296
His Asp Pro Phe Ala Gln Gln Arg Pro Tyr Glu Asn Phe Gln Asn Thr	
420 425 430	
GAG GGA AAA GGC ACT GTT TAT TCC AGT GCA GCC AGT CAT GGT AAT GCA	1344
Glu Gly Lys Gly Thr Val Tyr Ser Ser Ala Ala Ser His Gly Asn Ala	
435 440 445	
GTG CAC CAG CCC TCA GGG CTC ACC AGC CAA CCT CAA GTA CTG TAT CAG	1392
Val His Gln Pro Ser Gly Leu Thr Ser Gln Pro Gln Val Leu Tyr Gln	
450 455 460	
AAC AAT GGA TTA TAT AGC TCA CAT GGC TTT GGA ACA AGA CCA CTG GAT	1440
Asn Asn Gly Leu Tyr Ser Ser His Gly Phe Gly Thr Arg Pro Leu Asp	
465 470 475 480	
CCA GGA ACA GCA GGT CCC AGA GTT TGG TAC AGG CCA ATT CCA AGT CAT	1488
Pro Gly Thr Ala Gly Pro Arg Val Trp Tyr Arg Pro Ile Pro Ser His	
485 490 495	
ATG CCT AGT CTG CAT AAT ATC CCA GTG CCT GAG ACC AAC TAT CTA GGA	1536
Met Pro Ser Leu His Asn Ile Pro Val Pro Glu Thr Asn Tyr Leu Gly	
500 505 510	
AAT ACA CCC ACC ATG CCA TTC AGC TCC TTG CCA CCA ACA GAT GAA TCT	1584
Asn Thr Pro Thr Met Pro Phe Ser Ser Leu Pro Pro Thr Asp Glu Ser	
515 520 525	
ATA AAA TAT ACC ATA TAC AAT AGT ACT GGC ATT CAG ATT GGA GCC TAC	1632
Ile Lys Tyr Thr Ile Tyr Asn Ser Thr Gly Ile Gln Ile Gly Ala Tyr	
530 535 540	
AAT TAT ATG GAG AAT GGT GGG ACG AGT TCA TCA CTA CTA GAC AGC ACA	1680
Asn Tyr Met Glu Ile Gly Gly Thr Ser Ser Ser Leu Leu Asp Ser Thr	
545 550 555 560	
AAT ACG AAC TTC AAA GAA GAG CCA GCT GCT AAG TAC CAA GCT ATC TTT	1728
Asn Thr Asn Phe Lys Glu Glu Pro Ala Ala Lys Tyr Gln Ala Ile Phe	
565 570 575	
GAT AAT ACC ACT AGT CTG ACG GAT AAA CAC CTG GAC CCA ATC AGG GAA	1776
Asp Asn Thr Thr Ser Leu Thr Asp Lys His Leu Asp Pro Ile Arg Glu	
580 585 590	
AAT CTG GGA AAG CAC TGG AAA AAC TGT GCC CGT AAA CTG GGC TTC ACA	1824
Asn Leu Gly Lys His Trp Lys Asn Cys Ala Arg Lys Leu Gly Phe Thr	
595 600 605	
CAG TCT CAG ATT GAT GAA ATT GAC CAT GAC TAT GAG CGA GAT GGA CTG	1872
Gln Ser Gln Ile Asp Glu Ile Asp His Asp Tyr Glu Arg Asp Gly Leu	
610 615 620	
AAA GAA AAG GTT TAC CAG ATG CTC CAA AAG TGG GTG ATG AGG GAA GGC	1920
Lys Glu Lys Val Tyr Gln Met Leu Gln Lys Trp Val Met Arg Glu Gly	
625 630 635 640	
ATA AAG GGA GCC ACG GTG GGG AAG CTG GCC CAG GCG CTC CAC CAG TGT	1968
Ile Lys Gly Ala Thr Val Gly Lys Leu Ala Gln Ala Leu His Gln Cys	
645 650 655	
TCC AGG ATC GAC CTT CTG AGC AGC TTG ATT TAC GTC AGC CAG AAC	2013
Ser Arg Ile Asp Leu Leu Ser Ser Leu Ile Tyr Val Ser Gln Asn	
660 665 670	
TAA	2016

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 671 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gln Pro Asp Met Ser Leu Asn Val Ile Lys Met Lys Ser Ser Asp  
 1 5 10 15  
 Phe Leu Glu Ser Ala Glu Leu Asp Ser Gly Gly Phe Gly Lys Val Ser  
 20 25 30  
 Leu Cys Phe His Arg Thr Gln Gly Leu Met Ile Met Lys Thr Val Tyr  
 35 40 45  
 Lys Gly Pro Asn Cys Ile Glu His Asn Glu Ala Leu Leu Glu Glu Ala  
 50 55 60  
 Lys Met Met Asn Arg Leu Arg His Ser Arg Val Val Lys Leu Leu Gly  
 65 70 75 80  
 Val Ile Ile Glu Glu Gly Lys Tyr Ser Leu Val Met Glu Tyr Met Glu  
 85 90 95  
 Lys Gly Asn Leu Met His Val Leu Lys Ala Glu Met Ser Thr Pro Leu  
 100 105 110  
 Ser Val Lys Gly Arg Ile Ile Leu Glu Ile Ile Glu Gly Met Cys Tyr  
 115 120 125  
 Leu His Gly Lys Gly Val Ile His Lys Asp Leu Lys Pro Glu Asn Ile  
 130 135 140  
 Leu Val Asp Asn Asp Phe His Ile Lys Ile Ala Asp Leu Gly Leu Ala  
 145 150 155 160  
 Ser Phe Lys Met Trp Ser Lys Leu Asn Asn Glu Glu His Asn Glu Leu  
 165 170 175  
 Arg Glu Val Asp Gly Thr Ala Lys Lys Asn Gly Gly Thr Leu Tyr Tyr  
 180 185 190  
 Met Ala Pro Glu His Leu Asn Asp Val Asn Ala Lys Pro Thr Glu Lys  
 195 200 205  
 Ser Asp Val Tyr Ser Phe Ala Val Val Leu Trp Ala Ile Phe Ala Asn  
 210 215 220  
 Lys Glu Pro Tyr Glu Asn Ala Ile Cys Glu Gln Gln Leu Ile Met Cys  
 225 230 235 240  
 Ile Lys Ser Gly Asn Arg Pro Asp Val Asp Asp Ile Thr Glu Tyr Cys  
 245 250 255  
 Pro Arg Glu Ile Ile Ser Leu Met Lys Leu Cys Trp Glu Ala Asn Pro  
 260 265 270  
 Glu Ala Arg Pro Thr Phe Pro Gly Ile Glu Glu Lys Phe Arg Pro Phe  
 275 280 285  
 Tyr Leu Ser Gln Leu Glu Glu Ser Val Glu Glu Asp Val Lys Ser Leu  
 290 295 300  
 Lys Lys Glu Tyr Ser Asn Glu Asn Ala Val Val Lys Arg Met Gln Ser  
 305 310 315 320  
 Leu Gln Leu Asp Cys Val Ala Val Pro Ser Ser Arg Ser Asn Ser Ala  
 325 330 335  
 Thr Glu Gln Pro Gly Ser Leu His Ser Ser Gln Gly Leu Gly Met Gly  
 340 345 350  
 Pro Val Glu Glu Ser Trp Phe Ala Pro Ser Leu Glu His Pro Gln Glu  
 355 360 365  
 Glu Asn Glu Pro Ser Leu Gln Ser Lys Leu Gln Asp Glu Ala Asn Tyr  
 370 375 380  
 His Leu Tyr Gly Ser Arg Met Asp Arg Gln Thr Lys Gln Gln Pro Arg  
 385 390 395 400



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Gln Asn Val Ala Tyr Asn Arg Glu Glu Glu Arg Arg Arg Arg Val Ser  
405 410 415

His Asp Pro Phe Ala Gln Gln Arg Pro Tyr Glu Asn Phe Gln Asn Thr  
420 425 430

Glu Gly Lys Gly Thr Val Tyr Ser Ser Ala Ala Ser His Gly Asn Ala  
435 440 445

Val His Gln Pro Ser Gly Leu Thr Ser Gln Pro Gln Val Leu Tyr Gln  
450 455 460

Asn Asn Gly Leu Tyr Ser Ser His Gly Phe Gly Thr Arg Pro Leu Asp  
465 470 475 480

Pro Gly Thr Ala Gly Pro Arg Val Trp Tyr Arg Pro Ile Pro Ser His  
485 490 495

Met Pro Ser Leu His Asn Ile Pro Val Pro Glu Thr Asn Tyr Leu Gly  
500 505 510

Asn Thr Pro Thr Met Pro Phe Ser Ser Leu Pro Pro Thr Asp Glu Ser  
515 520 525

Ile Lys Tyr Thr Ile Tyr Asn Ser Thr Gly Ile Gln Ile Gly Ala Tyr  
530 535 540

Asn Tyr Met Glu Ile Gly Gly Thr Ser Ser Ser Leu Leu Asp Ser Thr  
545 550 555 560

Asn Thr Asn Phe Lys Glu Glu Pro Ala Ala Lys Tyr Gln Ala Ile Phe  
565 570 575

Asp Asn Thr Thr Ser Leu Thr Asp Lys His Leu Asp Pro Ile Arg Glu  
580 585 590

Asn Leu Gly Lys His Trp Lys Asn Cys Ala Arg Lys Leu Gly Phe Thr  
595 600 605

Gln Ser Gln Ile Asp Glu Ile Asp His Asp Tyr Glu Arg Asp Gly Leu  
610 615 620

Lys Glu Lys Val Tyr Gln Met Leu Gln Lys Trp Val Met Arg Glu Gly  
625 630 635 640

Ile Lys Gly Ala Thr Val Gly Lys Leu Ala Gln Ala Leu His Gln Cys  
645 650 655

Ser Arg Ile Asp Leu Leu Ser Ser Leu Ile Tyr Val Ser Gln Asn  
660 665 670

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45

What is claimed is:

1. An isolated RIP-Thr<sup>514</sup> polypeptide, comprising at least 10 consecutive amino acid residues of the amino acid sequence set forth as SEQ ID NO:2, which consecutive amino acid residues comprise the amino acid residue 514 (Thr) of SEQ ID NO:2.
2. An isolated polypeptide according to claim 1, wherein said polypeptide has an activity selected from at least one of: a kinase or kinase inhibitory activity or a RIP-binding or binding inhibitory activity.
3. A method of screening for an agent which modulates the interaction of a RIP polypeptide to a binding target, said method comprising the steps of:
  - incubating a mixture comprising:
    - an isolated polypeptide according to claim 1,
    - a binding target of said polypeptide, and
    - a candidate agent;
  - under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity;
  - detecting the binding affinity of said polypeptide to said binding target to determine an agent-biased affinity,

wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said polypeptide to said binding target.

4. A method according to claim 3, wherein said binding target is a natural intracellular substrate of the polypeptide and said agent-biased binding affinity is detected as phosphorylation of said substrate.

5. A method according to claim 3, wherein said binding target comprises a Tumor necrosis factor receptor Associated Factor-2 (TRAF2) or a Tumor necrosis factor Receptor-1 Associated Death Domain protein (TRADD).

6. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 1$  (SEQ ID NO:2, residues 509–518).

7. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 2$  (SEQ ID NO:2, residues 514–521).

8. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 3$  (SEQ ID NO:2, residues 506–514).

9. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 4$  (SEQ ID NO:2, residues 504–524).

## 17

10. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 5$  (SEQ ID NO:2, residues 498–514).

11. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 6$  (SEQ ID NO:2, residues 514–534).

12. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 7$  (SEQ ID NO:2, residues 513–520).

13. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 8$  (SEQ ID NO:2, residues 508–515).

14. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 9$  (SEQ ID NO:2, residues 512–522).

15. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 10$  (SEQ ID NO:2, residues 423–514).

16. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 11$  (SEQ ID NO:2, residues 423–543).

17. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 12$  (SEQ ID NO:2, residues 423–579).

## 18

18. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 13$  (SEQ ID NO:2, residues 423–633).

19. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 14$  (SEQ ID NO:2, residues 423–671).

20. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 15$  (SEQ ID NO:2, residues 514–543).

21. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 16$  (SEQ ID NO:2, residues 514–579).

22. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 17$  (SEQ ID NO:2, residues 514–633).

23. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise  $\alpha\Delta 18$  (SEQ ID NO:2, residues 514–671).

24. An isolated polypeptide according to claim 1, wherein said consecutive amino acid residues comprise SEQ ID NO:2.

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